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Synthesis, structure characterization and preliminary biological evaluation of novel 5-alkyl-2-ferrocenyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one derivatives

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Abstract

A series of novel 5-alkyl-2-ferrocenyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one derivatives were synthesized by the reaction of ethyl 1-(2-bromoethyl)-3-ferrocenyl-1*H*-pyrazole-5-carboxylate with non-aromatic primary amines in one-pot procedure and characterized by ^{1}H NMR, ^{13}C NMR, IR, HRMS and X-ray diffraction analysis. The effects of all the compounds on A549 cell growth were investigated. The results showed that all compounds had almost inhibitory effects on the growth of A549 cells.

Keywords: Ferrocene; Pyrazole-fused pyrazinone; Synthesis; X-ray structure; Bioactivity; A549 cell

1. Introduction

Since the discovery of ferrocene in 1951 [1], its fascinating sandwich structure has captured the imagination of chemists, to the point of being nowadays among the most important structural motifs in organometallic chemistry, materials science, and, especially, catalysis. Recently, ferrocene and its derivatives have been attracted much more attention in the biological activities [2,3]. Incorporation of a ferrocene fragment into a molecule of an organic compound often obtained unexpected biological activity, which is rationalized as being due to their different membrane permeation properties and anomalous metabolism [4]. Furthermore, the stability and non-toxicity of the ferrocenyl moiety is of particular interest rendering such drugs compatible with other treatment [5–7]. In this sense, the integration of one or more ferrocene units into a heterocyclic ring molecule has been recognized as an attractive way to endow a novel molecule functionally [8–12].

The pyrazole unit is one of the core structures in a number of natural products. Many pyrazole derivatives are known to exhibit a wide range of biological properties such as anti-hyperglycemic, analgesic, anti-inflammatory, antipyretic, anti-bacterial, antifungal hypoglycemic, sedative-hypnotic activity, antitumor and anticoagulant activity [13–15]. The incorporation of heterocyclic rings into prospective pharmaceutical candidates is a major tactic to gain activity and safety advantages. Although much work has been directed toward the design and synthesis of fused-pyrazole derivatives [16–21], a search of the literatures revealed very few reports concerning pyrazolo-pyrazinones [22]. In the previous papers, we synthesized a series of novel pyrazole derivatives including ethyl 1-(2′-hydroxy-3′-aroxypropyl)-3-aryl-1*H*-pyrazole-5-carboxylate derivatives [23], 6-(aroxymethyl)-2-aryl-6,7-dihydropyrazolo[5,1-

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c][1,4]oxazin-4-one derivatives [24], ethyl 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carboxylate derivatives [25], 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide derivatives [26] and 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives [27]. The evaluation of biological activity showed that these compounds can inhibit A549 lung cancer cell growth.

One would expect that the introduction of additional ferrocenilic fragments into molecules of pyrazoles of this class will afford products possessing a broader spectrum of useful biological characteristics. In our ongoing interest in the preparation of novel pyrazole derivatives, herein, we would like to report the synthesis, structure characterization and preliminary biological evaluation of a series of novel 5-alkyl-2-ferrocenyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one derivatives.

2. Results and discussion

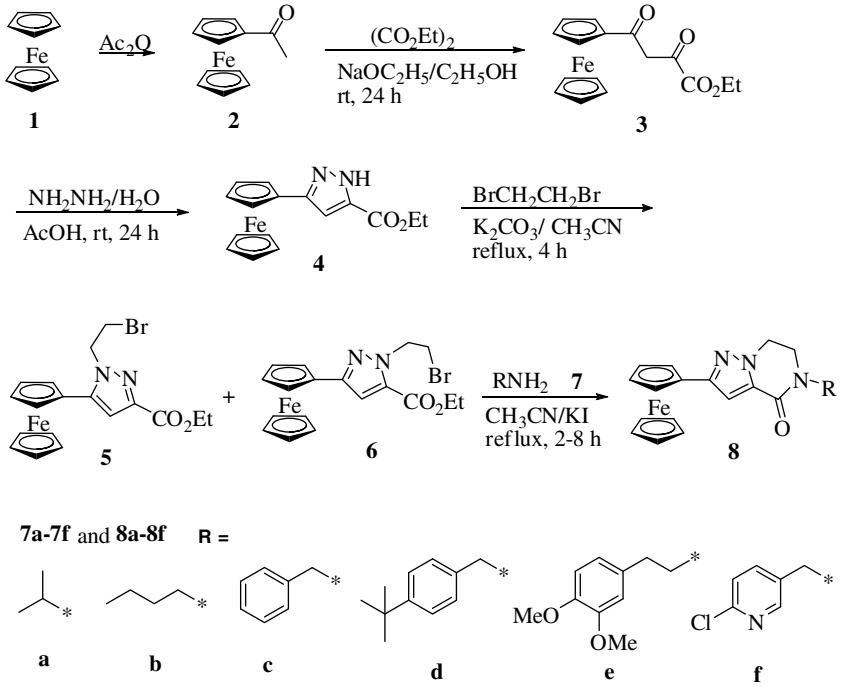
The synthetic strategies adopted to obtain the target compounds are depicted in Scheme 1. The key intermediate in the present study is the ethyl 1-(2-bromoethyl)-3-ferrocenyl-1*H*-pyrazole-5-carboxylate (**6**).

*2.1. Synthesis of ethyl 3-ferrocenyl-1H-pyrazole-5-carboxylate (**4**)*

Firstly, acetylferrocene (**2**) was prepared from ferrocene (**1**) and acetic anhydride with the catalytic phosphoric acid in 80% yield according to the known method [28]. It has proven to be successful that synthesis of ethyl 3-aryl-1*H*-pyrazole-5-carboxylate from ethyl 2,4-dioxo-4-arylbutanoate and hydrazine hydrate in the presence of acetic acid at room temperature [23]. Thus, ethyl 3-ferrocenyl-1*H*-pyrazole-5-carboxylate (**4**) was readily synthesized in 76% yield by the reaction of ethyl 2,4-dioxo-4-ferrocenylbutanoate (**3**), which can be obtained from acetylferrocene (**2**) and diethyl oxalate, with hydrazine hydrate in the presence of acetic acid at room temperature as shown in Scheme 1.

*2.2. Synthesis of ethyl 1-(2-bromoethyl)-3-ferrocenyl-1H-pyrazole-5-carboxylate (**6**)*

It is known that nitrogen atom in 1st position of a pyrazole moiety possesses nucleophilic ability to react with alkyl halide under a suitable condition [25]. The *N*-alkylation reaction between ethyl 3-ferrocenyl-1*H*-pyrazole-5-carboxylate (**4**) and excess 1,2-dibromoethane was achieved in the presence of potassium carbonate as the base in acetonitrile. After flash chromatography on silica gel, the ethyl 1-(2-bromoethyl)-3-ferrocenyl-1*H*-pyrazole-5-carboxylate (**6**) and the isomer, ethyl 1-(2-bromoethyl)-5- ferrocenyl-1*H*-pyrazole-3-carboxylate (**5**) were obtained in 57% and 25% yields, respectively (Scheme 1). It should be easily understood that owing to annular tautomerism, pyrazoles can exist in two tautomeric forms such as ethyl 3-ferrocenyl-1*H*-pyrazole-5-carboxylate (**4**) and ethyl 5-ferrocenyl-1*H*-pyrazole-3-carboxylate (**4′**) to lead two isomers of product. It should be noted that it was difficult to distinguish two isomers in general because there is a lack of specific spectroscopic data in the literature [12]. In present work, we differentiated successfully two isomers on X-ray diffraction analysis. The general view of the molecule **6** is given in Fig. 1 [29]. Thus, we can compare the chemical



Scheme 1.

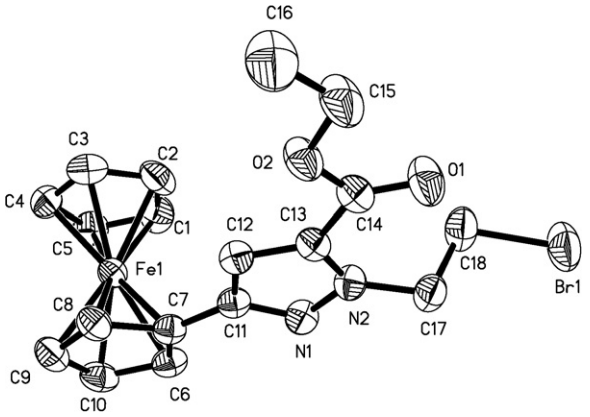
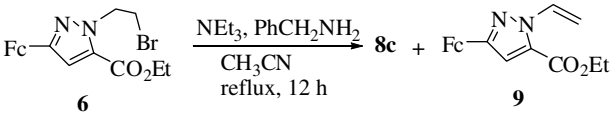


Fig. 1. The molecular structure of compound **6**, with displacement ellipsoids drawn at the 50% probability level and H atoms omitted.

shift of two isomers and the absolute value of chemical shift difference between two isomers. It should be found that C_{17}–H and two protons in substituted Cp of Fc moiety peaks in **6** are relatively downfield and appear around 4.92 and 4.69 ppm while corresponding protons in **5** are relatively upfield and resonate around 4.64 and 4.51 ppm. In contrast, five protons in the unsubstituted Cp (C_1–C_5) of Fc moiety peak in **6** is relatively upfield and appear around 4.08 ppm, while corresponding protons in **5** is relatively downfield and resonate around 4.20 ppm. Moreover, in the isomer **6**, the absolute value of chemical shift difference between protons in C_{17} and C_1–C_5 is mostly larger than that between respective protons in C_{17} and C_1–C_5 in isomer **5**, i.e. $|\Delta\delta$(H–H) isomer **6**$| > |\Delta\delta$(H–H) isomer **5**$|$ ($4.92 - 4.08 = 0.84 > 4.64 - 4.40 = 0.24$).

2.3. Synthesis of 5-alkyl-2-ferrocenyl-6,7-dihydropyrazolo[1,5-a]pyrazin-4(5H)-one (8)

Ethyl 1-(2-bromoethyl)-3-ferrocenyl-1*H*-pyrazole-5-carboxylate (**6**) reacted with excess non-aromatic primary amines **7** in acetonitrile to afford pyrazolo-pyrazinones derivatives **8** in moderate yields (Scheme 1). For example, **8b** was obtained in 76% yield by the reaction of **6** with excess *n*-butylamine **7b** (10 equiv.) that was indispensable as the substrate and base. To reduce the amount of reagent amine, we tried to use cheaper base, such as triethylamine. The result showed that the elimination reaction proceeded and afforded a by-product, which was assigned to be ethyl 3-ferrocenyl-1-vinyl-1*H*-pyrazole-5-carboxylate (**9**) by ^{1}H NMR. The ^{1}H NMR spectra indicated the presence of a double bond and the chemical shift of the double bond protons appeared at $\delta = 7.43$ ($J = 15.3$ and 9.7 Hz) in the form double doublet peaks, 6.01 ($J = 15.3$ Hz) and 5.04 ($J = 9.7$ Hz) ppm in the form of doublet peaks respectively, the coupling constants are consistent with *trans* and *cis* vinylic protons, respectively. The competition reaction resulted in the decrease of desired compound **8** (Scheme 2). On the other hand, the yield of compound **8** was depended on the structure of reagent amine **7**. From



Scheme 2.

Table 1, we can find that the more the steric hindrance of amine was, the lower the yield of compound **8**. For example, in the case of **8a** and **8b**, because the steric hindrance of isopropylamine is larger than *n*-butylamine, the yields of **8a** (23%) is less than **8b** (76%). Furthermore, as shown in Scheme 3, in the case of (*R*)-1-phenylethanamine, (*R*)-ethyl 3-ferrocenyl-1-(2-(1-phenylethylamino)ethyl)-1*H*-pyrazole-5-carboxylate (**10**) was only obtained, which could not undergo cyclization reaction in the same conditions to give desired compound.

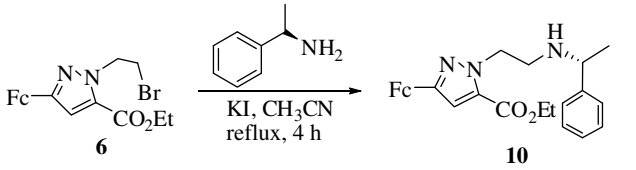
2.4. Crystal structure

The spatial structure of compound **8c** was determined by using X-ray diffraction analysis. The single crystals were grown from acetone at room temperature. The molecular view of **8c** is shown in Fig. 2, whereas Table 3 lists its selected bond lengths and angles.

Fig. 2 shows that **8c** contains a ferrocenyl bound to pyrazole ring which fused with pyrazinone. The molecular structure is dominated by the arrangement of the rings of the pyrazole, pyrazinone, two cyclopentadienyl of the ferrocenyl moiety and the phenyl. Steric hindrance of the protons in the C14 α-position of the Cp-ring and the proton at C12 and N3 of the pyrazole could be decreased by a perpendicular arrangement of the Cp and the pyrazole ring. In addition, an optimal electronic overlap of the π-system demands a coplanar arrangement. The dihedral angle between the substituted Cp ring (C14–C18) and the pyrazole ring (N2/N3/C10/C12/C13) of 16.08(11)° is less than that of the complex of ferrocenyl substituted pyrazolyl pyridine (20.5°) [30], but larger than that of **6** (8.20°). The C13–C14 distance 1.464(3) Å is same as that in **6** [29], but slightly shorter than that of substituted phenyl pyrazole derivatives (1.472(3) and 1.4785(19) Å) [31,32]. In the ferrocenyl moiety, the cyclopentadienyl (Cp) rings are perfectly planar but deviate slightly from being parallel, the angle between the plane normals being 1.66(13)°, and twisted from the eclipsed conformation by 13.41–14.00°. The angle Cg2–Fe1-Cg3 (where Cg is the centre of the plane defined by five C-atoms) is 178.16(5)°. The distances Cg2 –Fe and Cg3–Fe are 1.6420(13) Å and 1.6452(14) Å. The C–C bond lengths and C–C–C angles in the cyclopentadienyl rings show electronic overlap of the π-system-induced variations. The mean C–C distance involving the substituted C(14) atoms is the longest in the rings at 1.434(3) Å; adjacent C–C bonds are 1.424(3) and 1.416(3) Å, with the final C(16)–C(17) length being 1.422(3) Å. C–C–C angle at substituted atom averages 107.15(16)°, slightly reduced from the ideal 108°, with the other angles averaging

Table 1
Physical properties, HRMS and IR spectrum data for compounds **8**

Compound	Yield (%)	M.p. (°C)	Formula	HRMS: found	MS: require	IR (KBr) ν (cm^{-1})
8a	23	220–222	$C_{19}H_{21}FeN_3O$	$[M+H^+]$: 364.1113	364.1107	1650
8b	76	129–130	$C_{20}H_{23}FeN_3O$	$[M+H^+]$: 378.1264	378.1263	1650
8c	65	177–178	$C_{23}H_{21}FeN_3O$	$[M+Na^+]$: 434.0934	434.0926	1662
8d	42	214–215	$C_{27}H_{29}FeN_3O$	$[M+H^+]$: 468.1735	468.1733	1649
8e	65	180–181	$C_{26}H_{27}FeN_3O_3$	$[M+H^+]$: 486.1478	486.1475	1655
8f	34	230–232	$C_{22}H_1ClFeN_3O$	$[M+H^+]$: 447.0672	447.0670	1648



Scheme 3.

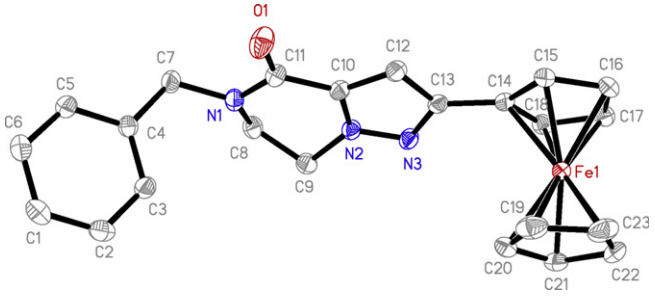


Fig. 2. The molecular structure of compound **8c**, with displacement ellipsoids drawn at the 50% probability level and H atoms omitted.

108.21°. Fe–C distances is longest for the substituted C(14), mean 2.0484(19) Å. All these bond and angle variations are very similar to those observed in **6**. In the pyrazole-fused pyrazinone ring, N1 and C11 are coplanar with pyrazole ring, forming π-system. The bond length of C10–C11 and C11–N1 are 1.471(3) and 1.357(3) Å, respectively (see Table 4).

2.5. Effects of the compounds on the viability of A549 lung cancer cells

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay is widely accepted as a reliable way to measure the cell proliferation rate, and conversely when metabolic events lead to apoptosis or necrosis. The data obtained by MTT assay showed that compounds **8a**–**8f** had inhibitory effects on the growth of A549 cells in dosage- and time-dependent manners as indicated by the results in Fig. 3. As typically shown in Fig. 3, exposure of cells to **8c**–**8f** at 20 μM for 24 h resulted in cell viability decrease from 100% to 64–51% ($p < 0.01$). When the exposure continued on to 48 h, compared with the control group, the cell viability reduced more significantly from 100% to 50–28% ($p < 0.01$). Further, exposure of cells to **8c**–**8f** at 40 μM for 24 and 48 h, the cell viability reduced more significantly from 100% to 43–33% and 34–17% ($p < 0.01$), respectively. To compare with known anticancer drug, we carried out the assay of the effects of 5-FU on the growth of A549 cells in the same conditions. From the IC_{50} of compounds **8a**–**8f** and 5-Fu we can find

Table 2
^1H NMR and ^{13}C NMR data for compound **8**

Compound	^1H NMR (300 MHz, δ, $CDCl_3$) and ^{13}C NMR (100 MHz, δ, $CDCl_3$)
8a	^1H NMR: 6.85 (s, 1H, PyzH), 5.08–4.95 (m, 1H, CH_3CH), 4.73 (s, 2H, C_5H_4), 4.34 (br s, 4H, C_5H_4 and NCH_2CH_2N), 4.11 (s, 5H, C_5H_5), 3.67 (s, 2H, NCH_2CH_2N), 1.23 (d, $J = 6.6$ Hz, 6H, $2CH_3$); ^{13}C NMR: 157.2, 151.5, 135.4, 104.8, 77.2, 69.5, 68.7, 66.5, 46.1, 43.4, 38.8, 19.7
8b	^1H NMR: 6.85 (s, 1H, PyzH), 4.72 (s, 2H, C_5H_4), 4.39–4.32 (m, 4H, C_5H_4 and NCH_2CH_2N), 4.12 (s, 5H, C_5H_5), 3.76 (t, $J = 6.3$ Hz, 2H, NCH_2CH_2N), 3.57 (t, $J = 7.2$ Hz, 2H, $NCH_2CH_2CH_2CH_3$), 1.70–1.57 (m, 2H, $NCH_2CH_2CH_2CH_3$), 1.47–1.33 (m, 2H, $NCH_2CH_2CH_2CH_3$), 0.97 (t, $J = 7.2$ Hz, 3H, CH_3); ^{13}C NMR: 157.8, 151.6, 135.4, 104.9, 77.2, 69.7, 68.9, 66.7, 46.2, 45.9, 45.5, 29.3, 20.1, 13.8
8c	^1H NMR: 7.37–7.27 (m, 5H, PhH), 6.93 (s, 1H, PyzH), 4.78 (s, 2H, $PhCH_2$), 4.70 (s, 2H, C_5H_4), 4.40–4.27 (m, 4H, C_5H_4 and NCH_2CH_2N), 4.10 (s, 5H, C_5H_5), 3.67 (t, $J = 6.3$ Hz, 2H, NCH_2CH_2N); ^{13}C NMR: 158.0, 151.7, 136.3, 135.0, 128.9, 128.2, 127.9, 105.1, 77.2, 69.5, 68.7, 66.5, 49.3, 45.8, 44.6
8d	^1H NMR: 7.38 (d, $J = 8.0$ Hz, 2H, PhH), 7.27 (d, $J = 8.0$ Hz, 2H, PhH), 6.92 (s, 1H, PyzH), 4.75–4.70 (m, 4H, C_5H_4 and $PhCH_2$), 4.32 (s, 4H, C_5H_4 and NCH_2CH_2N), 4.10 (s, 5H, C_5H_5), 3.68 (s, 2H, NCH_2CH_2N), 1.32 (s, CH_3, 9H); ^{13}C NMR: 158.0, 151.7, 150.9, 135.1, 133.2, 128.0, 125.8, 105.1, 77.2, 69.6, 68.7, 66.6, 49.1, 45.9, 44.6, 34.5, 31.3
8e	^1H NMR: 6.86 (s, 1H, PyzH), 6.84–6.77 (m, 3H, PhH), 4.70 (s, 2H, C_5H_4), 4.32 (s, 2H, C_5H_4), 4.20 (t, $J = 6.6$ Hz, 2H, NCH_2CH_2N), 4.10 (s, 5H, C_5H_5), 3.87 (s, 6H, OCH_3), 3.79 (t, $J = 7.2$ Hz, 2H, $PhCH_2CH_2$), 3.55 (t, $J = 6.6$ Hz, 2H, NCH_2CH_2N), 2.93 (t, $J = 7.2$ Hz, $PhCH_2$); ^{13}C NMR: 157.8, 151.6, 149.0, 147.7, 135.2, 131.1, 120.8, 111.8, 111.2, 104.8, 77.2, 69.5, 68.7, 66.5, 55.9, 55.8, 48.9, 46.6, 45.8, 33.8
8f	^1H NMR: 8.38 (s, 1H, PyH), 7.72 (dd, $J = 8.1$ and 1.8 Hz, 1H, PyH), 7.35 (d, $J = 8.1$ Hz, 1H, PyH), 6.90 (s, 1H, PyzH), 4.75 (s, 2H, $PhCH_2$), 4.73 (s, 2H, C_5H_4), 4.35 (s, 4H, C_5H_4 and NCH_2CH_2N), 4.11 (s, 5H, C_5H_5), 3.71 (br s, 2H, NCH_2CH_2N); ^{13}C NMR: 158.1, 152.0, 151.4, 149.3, 139.0, 134.6, 131.1, 124.7, 105.4, 77.2, 69.5, 68.8, 66.6, 46.5, 45.8, 45.1

Table 3
Selected bond lengths (Å) and angles (°) of compound **8c**

C(8)–N(1)	1.469(2)	C(14)–C(18)	1.430(3)
C(8)–C(9)	1.510(3)	C(14)–C(15)	1.434(3)
C(9)–N(2)	1.449(2)	C(14)–Fe(1)	2.0484(19)
C(10)–N(2)	1.352(2)	C(15)–C(16)	1.424(3)
C(10)–C(12)	1.377(3)	C(15)–Fe(1)	2.0423(19)
C(10)–C(11)	1.471(3)	C(16)–C(17)	1.422(3)
C(11)–O(1)	1.225(2)	C(16)–Fe(1)	2.039(2)
C(11)–N(1)	1.357(3)	C(17)–C(18)	1.416(3)
C(12)–C(13)	1.404(3)	C(17)–Fe(1)	2.038(2)
C(13)–N(3)	1.341(2)	C(18)–Fe(1)	2.038(2)
C(13)–C(14)	1.464(3)	N(2)–N(3)	1.342(2)
Cg(2)–Fe(1)	1.6420(13)	Cg(3)–Fe(1)	1.6452(14)
C(11)–N(1)–C(8)	121.56(16)	N(3)–C(13)–C(12)	110.83(16)
N(1)–C(8)–C(9)	112.25(16)	C(13)–N(3)–N(2)	105.01(14)
N(2)–C(9)–C(8)	107.75(15)	N(3)–N(2)–C(10)	112.48(15)
C(10)–N(2)–C(9)	122.57(16)	C(18)–C(14)–C(15)	107.15(16)
N(2)–C(10)–C(11)	121.15(16)	C(16)–C(15)–C(14)	108.25(16)
N(1)–C(11)–C(10)	115.39(16)	C(17)–C(16)–C(15)	107.77(17)
N(2)–C(10)–C(12)	106.59(16)	C(18)–C(17)–C(16)	108.42(17)
C(10)–C(12)–C(13)	105.08(16)	C(17)–C(18)–C(14)	108.39(17)
Cg(2)–Fe(1)–Cg(3)	178.16(5)		

Table 4
Crystal data and structure refinement for **8c**

Empirical formula	$C_{23}H_{22}FeN_3O$
Formula weight	412.29
Temperature (K)	293(2)
Wavelength (Å)	0.71069
Crystal system	Triclinic
Space group	$P\bar{1}$
Unit cell dimensions	
a (Å)	8.461(5)
b (Å)	10.473(5)
c (Å)	11.103(5)
α (°)	100.356(5)
β (°)	108.089(5)
γ (°)	93.568(5)
Volume (Å^3)	912.6(8)
Z	2
Calculated density (Mg/m^3)	1.500
Absorption coefficient (mm^{-1})	0.846
$F(000)$	430
Crystal size	0.27 × 0.16 × 0.10 mm
θ range for data collection (°)	1.97–27.49
Limiting indices	$-10 \leqslant h \geqslant 10$, $-13 \leqslant k \geqslant 13$, $-14 \leqslant l \geqslant 14$
Reflections collected/unique	13384/4168 [$R_{int} = 0.0220$]
Completeness to $\theta = 27.49°$	99.7%
Absorption correction	Semi-empirical from equivalents
Maximum and minimum transmission	0.9232 and 0.8044
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	4168/0/253
Goodness-of-fit on F^2	1.055
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0309$, $wR_2 = 0.0820$
R indices (all data)	$R_1 = 0.0378$, $wR_2 = 0.0863$
Largest diffraction peak and hole (e Å^3)	0.309 and −0.596

that compounds **8b**–**8f** have more effects on the growth of A549 cells then 5-Fu at 24 h. Furthermore, **8d**–**8f** have more effects then 5-Fu at 48 h (Table 5).

3. Summary

In summary, we have described the facile synthesis of novel 5-alkyl-2-ferrocenyl-6,7-dihydropyrazolo[1,5-*a*]pyrazin-4(5*H*)-one derivatives from ethyl 1-(2-bromoethyl)-3-ferrocenyl-1*H*-pyrazole-5-carboxylate and non-aromatic primary amines in one-pot procedure. The yield of the reaction was depended on the structure of reagent amine and base. These novel compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, HRMS and X-ray diffraction analysis. The results from effects of the compounds on the viability of A549 lung cancer cells showed that the compounds should be potential cancer inhibitor.

4. Experimental

All solvents were pre-dried and distilled prior to use. All reactions were carried out under nitrogen and monitored by TLC on silica gel 60 F_{254} plates (Merck KGaA). ^{1}H NMR spectra were recorded on a Bruker Avance 300 (300 MHz) spectrometer, using $CDCl_3$ as solvents and tetramethylsilane (TMS) as internal standard. Melting points were determined on an XD-4 digital micro melting point apparatus. IR spectra were recorded with an IR spectrophotometer Avtar 370 FT-IR (Termo Nicolet). MS spectra were recorded on a LTQ Orbitrap Hybrid mass spectrograph. X-ray diffraction data were recorded on a Bruker Smart Apex2CCD diffractometer. RPMI 1640 was obtained from Gibco BRL Co. (Grand Island, USA) and bovine calf serum was from Beijing DingGuo Biotechnology Co. (China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Amresco.

4.1. Synthesis of ethyl 3-ferrocenyl-1H-pyrazole-5-carboxylate (*4*) from (*2*)

The solution of acetylferrocene (1.14 g, 5.0 mmol) in absolute alcohol (2.5 ml) was added to a stirred solution of sodium ethoxide (408 mg, 6 mmol) and diethyl oxalate (877 mg, 6.0 mmol) in absolute alcohol (5.0 mL) (N_2 atmosphere). Stirring was continued for 24 h at room temperature, then glacial acetic acid (420 mg, 7 mmol) was added, and stirring was continued for an additional 30 min. 80% hydrazine hydrate (473 mg, 7 mmol) was added. Stirring was continued for 24 h at room temperature, then the mixture was combined with H_2O (30 mL) and the aqueous phase was extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were dried over anhydrate magnesium sulfate, and evaporated to give a yellow solid. Recrystallization from alcohol gave the product **4** (1.23 g, 76%) as yellow crystal. M.p. 196–198 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.47 (br s, 1H, NH), 6.84 (s, 1H, pyrazole moiety), 4.65 (s, 2H, C_5H_4), 4.42 (q, $J = 7.2$ Hz, 2H, OCH_2CH_3), 4.33 (s, 2H, C_5H_4), 4.09 (s, 5H, C_5H_5), 1.43 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3). IR (KBr) v: 1719 (C=O) cm^{-1}.

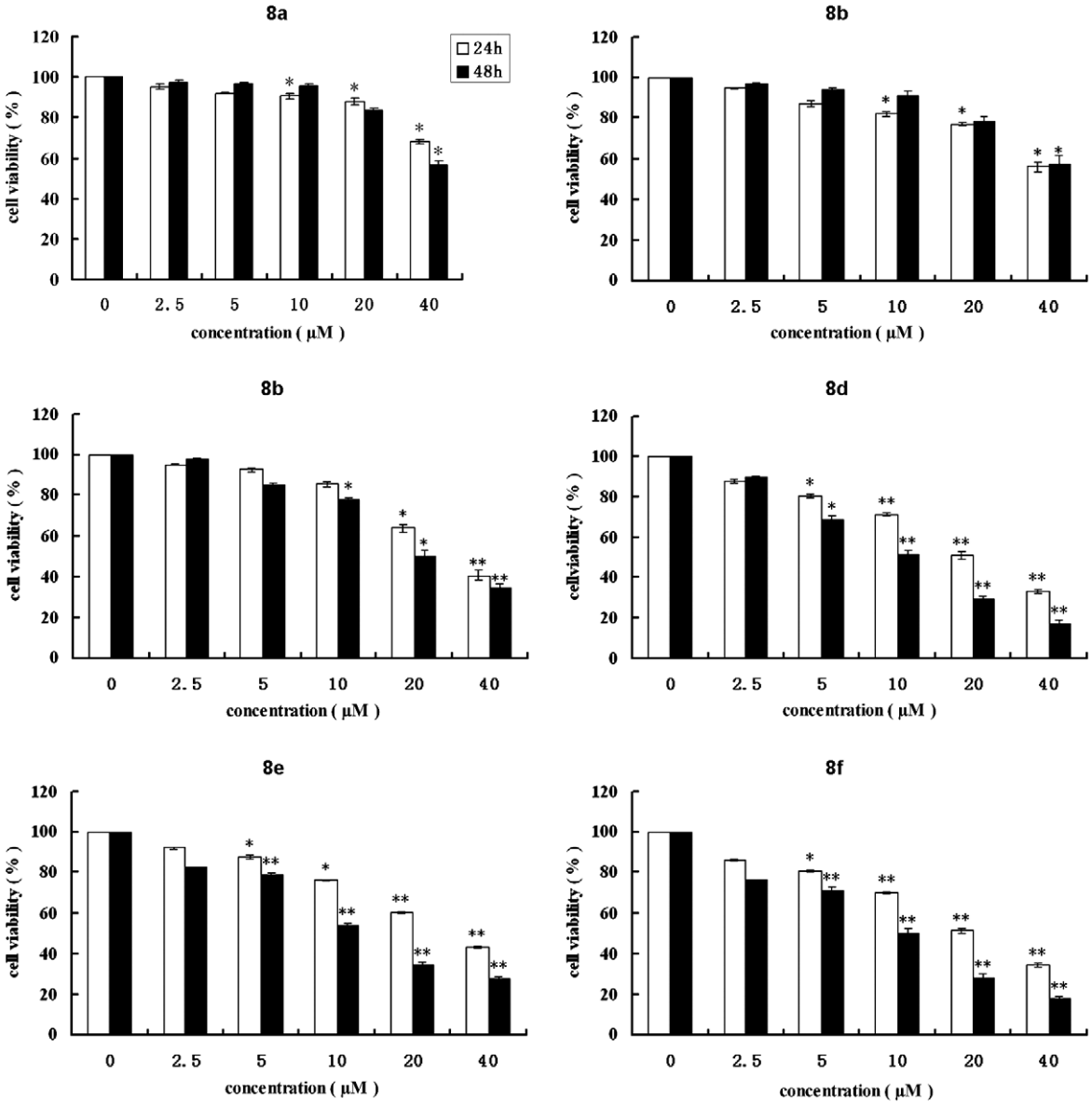


Fig. 3. Effects of the compounds **8a**–**8f** on the viability of A549 lung cancer cells. Control (0), the viability of the cells cultured in the medium without any compounds. Other bars show the viability of the cells treated with the compound **4a**–**4f** at the concentrations of 2.5–40 μM for 24 h and 48 h, respectively ($n = 3$).

Table 5
Growth inhibitory properties (IC_{50}) for the compounds **8a**–**8f** and 5-FU at 24 and 48 h

Compound	**8a**	**8b**	**8c**	**8d**	**8e**	**8f**	5-FU
24 h (μM)		51	30	22	31	22	
48 h (μM)	45	44	23	10	12	10	13

*4.2. Synthesis of ethyl 1-(2-bromoethyl)-3-ferrocenyl-1H-pyrazole-5-carboxylate (**6**) and ethyl 1-(2-bromoethyl)-5-ferrocenyl-1H-pyrazole-3-carboxylate (**5**)*

To a stirred solution of **4** (3.24 g, 0.01 mol) and 1,2-dibromoethane (18.79 g, 0.1 mol) in dry acetonitrile (40 mL) was added potassium carbonate (1.67 g, 0.012 mol) under N_2. After refluxed for 4 h, the reaction mixture was cooled to room temperature, filtered and evaporated, the residue was purified by chromatography on silica gel with the solvent system of ethyl acetate/petroleum ether ($v/v = 1:2$). The product **6** was obtained as yellow crystal in 57% yield. M.p. 98–99 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.85 (s, 1H, pyrazole moiety), 4.92 (t, $J = 6.9$ Hz, 2H, NCH_2CH_2Br), 4.69 (s, 2H, C_5H_4), 4.38 (q, $J = 7.2$ Hz, 2H, OCH_2CH_3), 4.30 (s, 2H, C_5H_4), 4.08 (s, 5H, C_5H_5), 3.74 (t, $J = 6.9$ Hz, 2H, NCH_2CH_2Br), 1.43 (t, $J = 7.2$ Hz, 2H, OCH_2CH_3). IR (KBr) ν: 1717 (C=O) cm^{-1}. The by-product **5** was obtained as a yellow crystal in 25% yield. M.p. 131–132 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.84 (s, 1H, pyrazole moiety), 4.64 (t, $J = 6.9$ Hz, 2H, NCH_2CH_2Br), 4.51 (s, 2H, C_5H_4), 4.43

(q, $J = 7.2$ Hz, 2H, OCH_2CH_3), 4.40 (s, 2H, C_5H_4), 4.20 (s, 5H, C_5H_4), 3.73 (t, $J = 6.9$ Hz, 2H, NCH_2CH_2Br), 1.42 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3). IR (KBr) v: 1711 (C=O) cm^{-1}.

4.3. General procedure for syntheses of pyrazolo-pyrazinones derivatives (*8*)

To a stirred solution of compound **6** (431 mg, 1.0 mmol) and amine **7** (10 mmol) in dry acetonitrile (20 mL) was added catalytic KI (33 mg, 0.2 mmol) under N_2. After refluxed for 2–8 h, the reaction mixture was cooled to room temperature, filtered and evaporated. The residue was purified by chromatography on silica gel with the solvent system of ethyl acetate/petroleum ether ($v/v = 1:1$) to afford desired derivatives **8** in moderate yields. The physical properties, Mass analysis data, IR spectrum data and ^1H NMR spectra of compounds **8** were listed in Tables 1 and 2, respectively.

4.4. Synthesis of (*R*)-ethyl 3-ferrocenyl-1-(2-(1-phenylethylamino)ethyl)-1*H*-pyrazole-5-carboxylate (*10*)

To a stirred solution of compound **6** (431 mg, 1.0 mmol) and (*R*)-1-phenylethanamine (1.21 g, 10 mmol) in dry acetonitrile (20 mL) was added catalytic KI (33 mg, 0.2 mmol) under N_2. After refluxed for 4 h, the reaction mixture was cooled to room temperature, filtered and evaporated. The residue was purified by chromatography on silica gel with ether to give **10** (316 mg, 67%) as yellow oil. ^1H NMR (300 MHz, $CDCl_3$): δ 7.40–7.20 (m, 5H, PhH), 6.83 (s, 1H, pyrazole moiety), 4.68–4.63 (m, 4H, C_5H_4 and NCH_2CH_2N), 4.33 (q, $J = 7.2$ Hz, 2H, OCH_2CH_3), 4.27 (s, 2H, C_5H_4), 4.05 (s, 5H, C_5H_5), 3.81 (q, $J = 6.6$ Hz, 1H, CH), 2.93 (t, $J = 6.0$ Hz, 2H, NCH_2CH_2N), 2.29 (br s, 1H, NH), 1.38 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 1.34 (d, $J = 6.6$ Hz, 3H, CH_3); IR (film) v 1719.8 cm^{-1}; HRMS (FT-ESI) m/z $[M+H]^+$ calc for $C_{26}H_{30}FeN_3O_2$: 472.1682. Found: 472.1674%.

4.5. Cell culture

A549 lung cancer cells were cultured in RPMI 1640 medium, supplemented with 10% (v/v) newborn calf serum at 37 °C in 5% CO_2, and 95% air. The cells were routinely seeded at the density of 1000/cm^2 into 96-well plates or other appropriate dishes containing the medium.

4.6. MTT assay for cell viability

The compounds were dissolved in DMSO. The final concentration of DMSO was below 0.1% in the culture medium (v/v) (DMSO at these final concentrations did not affect the viability of the cells). Cells were seeded in 96-well plates and treated with compounds **8a**–**8f** and 5-FU 2.5–40 μM for 24 and 48 h, respectively. The cell viability was determined by the MTT assay following the procedure described by Price and McMillan [33]. The light absorptions were measured at 570 nm using SpectraMAX 190 microplate spectrophotometer (GMI Co., USA).

4.7. Statistical analyses

Data were expressed as means ± SE, accompanied by the number of experiments performed independently, and analyzed by t-test. Differences at $p < 0.05$ were considered statistically significant.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2008.01.043.

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